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NOTES

Methods Used to Monitor the Microbial Load of Returned Lunar Material

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Methods employed to prepare lunar soil samples and procedures used to detect viable microorganisms are described.

Samples returned from the lunar surface were analyzed for the presence of viable microorganisms under strict quarantine conditions to prevent possible contamination of the earth's biosphere. All operations were performed under sterile conditions within class III biological glove cabinetry (S. Blickman, Weehawken, N.J.). The cabinetry was maintained at a negative pressure of 1 inch of water to minimize outflow of potentially hazardous material in the event of an accidental compromise in the barrier system.

The lunar materials obtained during the first two manned lunar-landing missions were of two types, based upon the area of collection. The two sample types consisted of a surface sample and a subsurface sample that were tested independently and were never mixed. The subsurface (or core) samples consisted of finely powdered lunar material with the majority of particles less than 100 μm in diameter. The surface samples contained a combination of fine material scooped from the Apollo lunar sample-return containers and rock chips obtained during the preliminary geological examinations.

The individual particles of the lunar samples were reduced to a common size range before inoculation into microbiological media. Each sample was separated through a laboratory sieve with a mean pore size of 104 μm. Particles larger than 104 μm were ground with a motor-driven sample crusher (model 150, LeMaire Instruments, Reno, Nev.). After recombination, all materials were separated through a sieve with a mean pore size of 40 μm.

Particles larger than 40 μm were ground to a mean diameter of 1.5 μm with an electric mortar grinder (Torsion Balance Co., model MG-2). Smaller material was not ground. A riffle sampler was used to homogenize and separate into fractions the resulting composite of ground and unground material. The final mean diameter of particles used for inoculation into the test systems was between 1.5 and 2.0 μm with particles as large as 40 μm in diameter being present.

Portions of each category of lunar material were cultured with the addition of molten (60 C), 3% purified agar (BioQuest; Fig. 1). No nutrient or energy source was added to this culture system. Each petri plate, containing 223 mg of lunar material in 10 ml of agar, was incubated, along with an uninoculated control, at one of the temperature/atmosphere combinations shown. No growth was observed on any of the plates throughout the 21-day incubation period.

For all other test systems, the lunar material was suspended in sterile 0.1 M phosphate buffer (*p*H 7.0) and filtered through Whatman no. 3 filter paper. Both the filtrate and the residue were divided into samples, representing wash and residue from 223 mg of lunar material. Blood agar (Blood Agar Base, BioQuest; 5% sheep blood, Colorado Serum Co.), Trypticase Glucose Yeast Extract Agar (BioQuest), and Thioglycolate Broth (BioQuest) were individually inoculated with the wash and residue samples (Fig. 2). In addition, Czapek Dox Agar (BioQuest) and Sabouraud Dextrose Agar (Difco), were inoculated with portions from the two surface samples. Each of these five nutrient media was used in both the recommended concentrations and in a dilution of 1:100 the normal nutrient concentration. Portions from each of the two surface

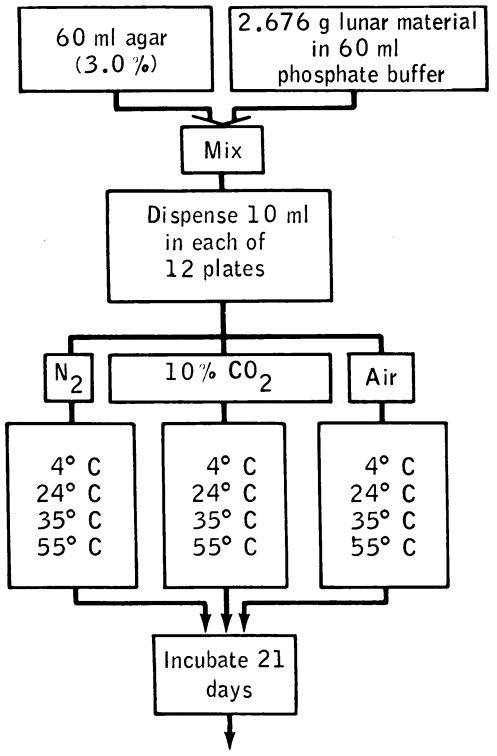

FIG. 1. *Microbiology protocol for unenriched lunar sample.*

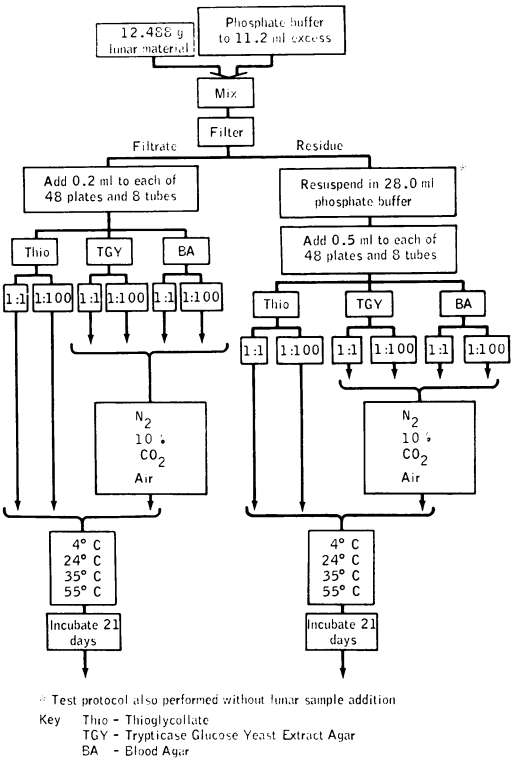

FIG. 2. *Microbiology protocol for inoculation of subsurface samples onto enriched media.*

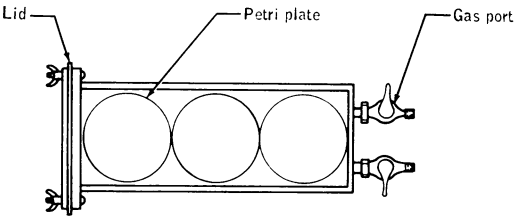

FIG. 3. *Transparent controlled-environment enclosure.*

samples were also transferred to a terrestrial soil extract in agar and to six different aquatic-mud media.

All inoculated solid media, with the corresponding uninoculated controls, were incubated in the atmosphere-temperature combinations shown in Fig. 1 and 2. For this purpose, the plates, in groups of three, were placed into a specially designed controlled-environment enclosure (Fig. 3). The enclosures were purged three times with the appropriate filter-sterilized gas for N_2 or 10% CO_2 (in air) incubation. All plates were incubated for 21 days and were observed periodically for growth. Growth was not observed on any of these enriched media.

The lack of growth in any of these test systems may be interpreted in at least three ways: (i) the lunar material was free of viable microorganisms, (ii) viable microorganisms were present but inhibited by the lunar materials, or (iii) viable obligate autotrophs were present but their growth was not supported in the culture media employed. Additional testing is required to evaluate these and other interpretations.